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MEAT PRESERVATION

Oxidative Changes in Cured and Uncured Frozen Cooked Pork

MARELYNN W. ZIPSER,
TAI-WAN KWON¹, and
BETTY M. WATTS

Department of Food and Nutrition,
Florida State University,
Tallahassee, Fla.

A PRIMARY OBJECTIVE of this paper is a comparison of patterns of lipid oxidation in frozen cooked meats versus frozen cured meats. Recent work from this laboratory on the freezing preservation of roast beef slices (2) and of pre-cooked mullet (18) reveals a similar pattern of lipid oxidation as measured by the thiobarbituric acid (TBA) test.

¹ Present address: Department of Nutritional Science, College of Agriculture, University of California, Berkeley, Calif.

Both the meat and fish had moderately high TBA numbers throughout the period of frozen storage, with no significant changes related to storage time. The results were interpreted as a rapid oxidation of the meat lipids during preparation for freezing and upon subsequent thawing. The oxidation was believed to be catalyzed by the ferric cooked meat pigment. The reaction evidently was arrested in the freezer, but proceeded rapidly upon thawing.

The addition of sodium nitrite and salt to meat before heat treatment would be expected to change this pattern. Nitrite converts the meat pigments to the catalytically inactive ferrous nitric oxide hemochromogen; consequently, the stability of refrigerated cured meats to lipid oxidation is high as compared to the stability of cooked meats under the same conditions (77). On the other hand, sodium chloride used in curing brines is known to accelerate lipid

The pattern of lipid oxidation in cured vs. uncured frozen cooked pork differed markedly. Heme-catalyzed oxidation during preparation for freezing and thawing, but not during frozen storage, appeared responsible for changes in uncured meat. Cured samples exhibited salt-catalyzed oxidation during frozen storage. The ratio of peroxides to TBA number was 8 to 10 times as high in cured as in uncured meats. This is attributed to the more rapid decomposition of peroxides by the ferric hemes of cooked meat. Rancid odor showed a highly significant correlation (Spearman $r_s = 0.92$) with TBA number in both cured and uncured meat. Pigment losses in cured samples were correlated with both peroxides and TBA number ($r_s = 0.87$). No completely satisfactory antioxidant was found for frozen cured meat.

oxidation under conditions of low free moisture, as would occur in frozen foods where the water is converted into ice (7). Cured meats are notoriously subject to oxidative changes when stored in the freezer.

The cured meat pigment is easily destroyed by fat peroxides, and pigment losses have been shown to parallel lipid oxidation in refrigerated hams (74). Similar discolorations would be expected in frozen cured meats. Therefore, measurements of cured meat pigment concentrations are included in the present study.

Although the heme-catalyzed oxidations in cooked meats can be easily prevented by the addition of polyphosphates and vegetable extracts (10), no satisfactory antioxidants have been reported for the protection of cured meats stored in the freezer. The effectiveness of several antioxidants has been determined in this study.

Methods

Preparation of Meat. Fresh pork ham was used exclusively. The meat was trimmed of excess fat and ground twice in Wards' Electric Food Chopper, Model VGS-5169A. The ground meat was mixed thoroughly by hand to obtain a homogeneous product. After addition of the appropriate additives, the meat was thoroughly mixed and weighed into No. 2 cans which were sealed immediately. The cans of meat were heated with free-flowing steam in an autoclave until an internal temperature of 70° C. was reached. The cans were cooled in a running water bath. They were then opened and the contents ground using attachments for the Kitchen-Aid mixer, Model 13-C. One-hundred-gram portions of the ground cooked meat were weighed and pressed into a uniform shape using a hamburger press. The meat was placed in polyethylene bags which were heat sealed and stored at 0° F. (-18° C.). Before assay, samples were thawed by immersing the packages in hot tap water (49° C.) for 25 minutes.

Lipid Oxidation. The 2-thiobarbituric acid (TBA) test (72) was used to measure the malonaldehyde (a lipid

Table I. The Effects of Curing Salts and Antioxidants on Frozen Cooked Pork

Storage Time, Weeks	Sample Description	TBA No. ^a	Peroxide No.	Cured Meat Pigment, % Retention ^b	Average Sensory Score	Significant Difference in Scores
0	Uncured-plain	0.7	
	Uncured-antiox.	0.1	
	Cured-plain	0.0	...	100	...	
	Cured-antiox.	0.0	...	100	...	
13	1 ^c Uncured-plain	4.1	3.1	
	2 Uncured-antiox.	0.0	5.4	2, 4 > 1, 3
	3 Cured-plain	2.5	...	27.6	2.6	
	4 Cured-antiox.	0.1	...	86.5	5.8	
23	1 Uncured-plain	5.5	4.0	...	3.3	
	2 Uncured-antiox.	0.0	3.5	...	5.7	2, 4 > 1, 3
	3 Cured-plain	4.1	45.7	6.4	2.7	
	4 Cured-antiox.	1.4	20.0	70.5	5.4	
47	1 Uncured-plain	4.2	5.9	...	3.0	
	2 Uncured-antiox.	0.0	3.5	...	5.4	2 > 1, 3, 4 3 > 4 1 > 3
	3 Cured-plain	5.9	91.1	trace	1.9	
	4 Cured-antiox.	6.8	126.0	approx. 10	3.6	

^a TBA numbers for initial and 13 week assays obtained by standard TBA method. Modified method used for assay of cured samples in subsequent storage periods.

^b Based on a hematin concentration of 45 p.p.m. in freshly cured sample. Only 65% of total pigment was converted to the nitroso form.

^c Numbers preceding sample description are used to indicate statistical significance of sensory scores (right column).

Table II. Effect of Salt and Nitrite on Frozen Pork

Storage Time, Weeks	Sample	TBA No.	Peroxide No.	Pigment Ratio 570 mμ to 650 mμ ^a	Average Sensory Score	Significant Difference in Scores
8	1 ^b Plain	4.8	10.3	...	2.6	4 > 1, 3
	2 Cured	0.8	13.4	2.35 ^c	5.3	
	3 NaCl	12.9	26.0	...	1.9	1 > 3
	4 NaNO ₂	0.5	13.4	2.55 ^c	5.4	
16	Plain	6.5	6.4	...	2.8	All highly significant
	Cured	1.9	13.3	2.01	4.3	
	NaCl	13.3	23.4	...	1.4	
	NaNO ₂	1.1	5.8	2.30	5.3	
27	1 Plain	5.8	9.4	...	3.8	4 > 2, 3
	2 Cured	8.5	61.1	1.40	1.7	
	3 NaCl	17.5	43.1	...	1.7	
	4 NaNO ₂	1.8	13.7	2.19	5.4	
51	1 Plain	7.6	10.5	...	3.7	4 > 1, 2, 3 1 > 2, 3
	2 Cured	9.8	60.0	1.45	1.8	
	3 NaCl	15.2	34.3	...	1.3	
	4 NaNO ₂	2.4	21.6	2.01	5.0	
66	Plain	7.0	12.5	
	Cured	8.1	120.7	1.26	...	
	NaCl	12.6	25.6	
	NaNO ₂	2.5	16.6	1.99	...	

^a In fresh cured meats, average ratio is 2.59. Falls to a range of 1.3-1.6 when pigment is oxidized (3).

^b Same as footnote c Table I.

^c Initial ratios: cured, 2.54; NaNO₂, 2.77.

Table III. Effect of KCl and NaCl Concentration on Rancidity in Frozen Pork

Storage Time, Weeks	Sample	TBA No.	Peroxide No.	Average Sensory Score	Significant Differences in Scores
6	1 ^a Plain	3.8	6.4	4.0	4 > 2, 3
	2 NaCl, 1%	7.6	10.2	2.7	
	3 NaCl, 4%	9.4	5.5	2.3	
	4 KCl	1.7	9.9	4.6	
23	1 Plain	4.7	3.2	3.6	1, 4 > 2, 3
	2 NaCl, 1%	9.5	7.7	1.9	
	3 NaCl, 4%	14.9	19.5	1.1	
	4 KCl	2.5	2.5	4.1	
38	1 Plain	3.8	3.1	3.1	1, 4 > 2, 3
	2 NaCl, 1%	7.8	12.3	1.7	
	3 NaCl, 4%	11.8	12.6	1.3	
	4 KCl	1.6	2.2	3.6	
52	1 Plain	3.9	3.7	4.2	1, 4 > 2, 3
	2 NaCl, 1%	9.9	8.5	2.2	
	3 NaCl, 4%	13.5	12.8	2.0	
	4 KCl	1.8	3.4	4.7	

^a Same as footnote c, Table I.

Table IV. Antioxidants in Frozen Cured Pork

Storage Time, Days	Sample	TBA No.	Peroxide No.	Pigment Ratio 570 mμ to 650 mμ	Average Sensory Score	Significant Difference in Scores
0	Control	0	0.9	2.37	...	
	Tripoly.	0	1.1	2.42	...	
	Onion	0	0.6	2.47	...	
	Pepper	0	2.1	2.36	...	
	BHA	0	1.4	2.51	...	
	Trip. + asc.	0	1.4	2.47	...	
30	1 ^a Control	1.3	18.6	2.28	4.3	6 > 1
	2 Tripoly.	1.7	13.5	2.25	5.0	
	3 Onion	0.4	14.6	2.34	5.0	
	4 Pepper	0.4	12.3	2.19	5.3	
	5 BHA	0.1	12.5	2.28	5.3	
	6 Trip. + asc.	0.2	16.0	2.25	5.9	
97	1 Control	3.8	29.6	1.84	3.0	5, 6 > 1, 2, 3, 4
	2 Tripoly.	2.4	22.5	2.05	3.7	
	3 Onion	6.6	49.6	1.63	3.2	
	4 Pepper	3.8	31.7	1.92	3.6	
	5 BHA	0.2	18.0	2.41	5.6	
	6 Trip. + asc.	0	17.4	2.13	5.9	
192	1 Control	7.0	62.2	1.48	2.3	
	5 BHA	0.3	11.5	2.00	5.1	
	6 Trip. + asc.	0.1	10.5	1.97	5.5	
370	1 Control	9.1	68.6	1.35	2.1	5 > 6, 1
	5 BHA	0.6	20.3	1.85	5.1	
	6 Trip. + asc.	13.7	81.7	1.36	1.9	

^a Same as footnote c, Table I.

Table V. Ascorbate and Tripolyphosphate as Antioxidants for Frozen Cured Pork

Storage Time, Days	Sample	TBA No.	Peroxide No.	Pigment Ratio 570 mμ to 650 mμ	Average Sensory Score	Significant Difference in Scores
17	Control	0.5	11.1	2.37	...	
	Asc.	0.3	8.7	2.52	...	
	Tripoly.	0.4	7.4	2.41	...	
	Trip. + asc.	0.3	6.7	2.30	...	
85	Control	3.4	31.3	2.00	...	
	Asc.	1.0	11.0	2.44	...	
	Tripoly.	1.0	10.6	2.39	...	
	Trip. + asc.	0.1	6.5	2.42	...	
154	Control	3.5	32.6	2.20	4.9	None
	Asc.	1.8	18.1	2.41	5.0	
	Tripoly.	1.8	25.0	2.33	5.4	
	Trip. + asc.	0.8	11.0	2.56	5.6	
208	1 ^a Control	6.6	59.1	1.56	2.5	3 > 1
	2 Asc.	2.6	19.9	2.19	5.6	
	3 Tripoly.	4.7	28.9	1.92	3.9	
	4 Trip. + asc.	1.7	14.7	2.23	5.1	

^a Same as footnote c, Table I.

oxidation product) which could be distilled from the meat samples. Since nitrite interferes in this test, a modified procedure (19) was used for cured samples. In both procedures, duplicate determinations were made and the average was reported as the TBA number—i.e., milligrams of malonaldehyde per 1000 grams of meat.

Peroxides in carbon tetrachloride extracts of the tissue were determined by a modification of the Wheeler method (16). Duplicate determinations were made and the average was reported as peroxide number—i.e., milliequivalent of peroxide per 1000 grams fat.

Pigment Changes. The concentration of unoxidized cured meat pigment was determined by the acetone extraction procedure of Hornsey (7) as modified by Gantner (6). Duplicate determinations were made. In other experiments, oxidation of the cured meat pigment was followed by reflectance measurements (3).

Organoleptic Evaluation. The intensity of rancid odor was rated by a panel of trained judges as described by Tarladgis *et al.* (13). Together seven to ten judges rated the samples at any one storage period. Numerical values ranging from 6 (no rancid odor) to 1 (very strong rancid odor) were assigned the judgments, and the average score was calculated for each sample. The Wilcoxon matched-pairs, signed-ranks test was applied to evaluate the significance of differences. Differences are indicated as significant at a level of 0.05 or less.

Other Tests. Qualitative tests for nitrite and sulfhydryl groups, both known to be important in the retention of cured meat color, were carried out as outlined by Erdman and Watts (5).

Experimental Results

Experiment 1 compares lipid oxidation and pigment loss in cured *vs.* uncured frozen pork. The effect of the antioxidant combination of sodium tripolyphosphate and sodium ascorbate on both cured and uncured samples was also studied. Ground mixed pork was divided into four batches which were treated as follows: no antioxidants or curing salts; antioxidants but no curing salts; curing salts but no antioxidants; and both curing salts and antioxidants. The proportions of antioxidants were 0.5% sodium tripolyphosphate and 0.22% sodium ascorbate. The curing salts were 2% sodium chloride and 0.03% sodium nitrite. The results of analyses performed at intervals during a year of frozen storage are shown in Table I.

Experiment 2 assesses the over-all effects of salt and nitrite, separately and together, in frozen pork. Two per cent NaCl and 0.03% NaNO₂ were added to portions of the same lot of mixed ground pork to obtain samples containing no

additive salt alone nitrite alone and both salt and nitrite. The data are shown in Table II.

Experiment 3 was designed to determine if differences in the initial salt concentration have any effect on the rate of lipid oxidation in uncured frozen meat. The concentrations investigated were 1 and 4% NaCl. KCl, in a concentration of 5.1% (equimolar to 4% NaCl), was also tried. Chang and Watts (7) found that NaCl had a much greater catalytic effect than KCl on lipid oxidation in an artificial aqueous fat system. The data are presented in Table III.

Experiment 4 tests the effectiveness of various antioxidants in preventing lipid oxidation and pigment loss in frozen cured meat. Salt (4%) and NaNO₂ (0.03%) were added to the ground pork. Separate batches of the meat containing the curing salts were further treated as follows: control (no additional additive); tripoly (0.1% tripolyphosphate); onion (hot water soluble material from 1 gram of green onion tops in 100 grams of meat); pepper (hot water soluble material from 1 gram of green pepper in 100 grams of meat); BHA (0.01% butylated hydroxyanisole, added in the form of Sustane E, Universal Oil Products Co.); and tripoly + asc. (0.1% tripolyphosphate + 0.1% sodium ascorbate). The results are shown in Table IV.

In experiment 5, summarized in Table V, ascorbate and tripolyphosphate were added to cured pork separately as well as in combination. The concentrations used were 0.1% ascorbate and 0.5% tripolyphosphate.

Discussion

Pattern of Lipid Oxidation in Cured vs. Uncured Samples without Antioxidants. The TBA numbers of untreated cooked pork (Tables I, II, and III) show the same pattern described in previous studies with frozen roast beef

slices (2) and mullet (78). The values are well above the sensory threshold (believed to be approximately 1.0) at the first storage period but show no progressive increase during storage.

The TBA numbers of cured meats, on the other hand, are initially negligible, but increase progressively throughout storage (Tables I, II, IV, and V). Heme catalysis during freezing and thawing is eliminated by conversion to the cured meat pigment brought about by nitrite, but salt accelerates lipid oxidation in the freezer.

When nitrite alone is added (Table II), the cured meat pigment is fully developed and TBA numbers throughout storage are lower than in corresponding cooked or cured meats. The addition of salt without nitrite results in both heme and salt catalysis, with very high TBA numbers. Increasing the salt concentration from 1 to 4% (Table III) resulted in higher TBA numbers, but even the lower concentration brought about marked increases in lipid oxidation as compared to the unsalted control.

Potassium chloride in the same molar concentration as 4% sodium chloride not only did not accelerate lipid oxidation but actually gave some protection as compared to the control sample (Table III). Chang and Watts (7) noted that this salt lacked the marked effect on lipid oxidation shown by sodium chloride and calcium chloride. The observation is not of practical importance, since the bitter taste of the potassium salt would preclude its use in foods.

In comparing peroxide numbers with TBA numbers, several factors must be kept in mind. Peroxide numbers are determined on lipids (mainly triglycerides) extracted from the tissue with carbon tetrachloride. The oxidation of phospholipids or protein-bound lipids would not be measured. On the other hand, the TBA test is applied to distillates from the entire meat sample and should, therefore, measure malonaldehyde from all lipids. Also, peroxides are

intermediates in the oxidative decomposition of unsaturated fatty acids, whereas malonaldehyde is an end product. In the presence of strong peroxide decomposers, such as metals or heme compounds, a lowered ratio of peroxide to malonaldehyde might be expected.

Ratios of peroxide to TBA numbers have been calculated for cured and uncured meats, with and without various additives. The results are summarized in Table VI. The ratios for uncured meats are strikingly low as compared to those for cured meats—i.e., very little peroxide can be extracted from uncured frozen meats as compared to the amount obtained from cured samples at the same TBA level. Similar results were interpreted earlier as evidence that heme-catalyzed oxidation in the cooked meats is largely confined to lipid fractions not extracted by carbon tetrachloride, whereas salt catalyzes oxidation in the triglycerides which are extracted in the peroxide test (75).

The additional data now available on uncured salted samples throw doubt on this interpretation. The addition of salt to uncured meat did not increase the ratio of peroxide to TBA numbers significantly. Furthermore, meats containing nitrite but no salt showed the same high peroxide to TBA ratio as the cured, salted samples. The interpretation that peroxides cannot accumulate in cooked meats because their rapid decomposition by the ferric pigments is more consistent with the facts.

Antioxidants. In this, as in earlier work from this laboratory, tripolyphosphate plus ascorbate eliminated the heme-catalyzed lipid oxidation of uncured cooked meats (Table I). Tripolyphosphate alone, even at much lower concentrations, has proved effective (70). Tripolyphosphate also protects refrigerated cured meats (74).

This was not true of frozen cured meats. Tripolyphosphate alone, at concentrations of 0.1% (Table IV) an

Table VI. Ratio of Peroxide Number to TBA Number

Sample Composition	No. of Samples	Peroxide:TBA	
		Range	Av.
Uncured, no addition	13	0.68-2.8	1.4
Uncured, salt added	12	0.59-2.5	1.6
Cured, no antioxidant	25	5.0-27	11
Cured, unsalted	7	5.3-27	10.4
Cured, tripoly.	10	8.0-21	13
Cured, BHA or tripoly + ascorbate	14

^a Calculation meaningless. Ratios very high in early storage periods. TBA numbers generally ranged from 0 to 0.2, and peroxides from 10 to 20.

Table VII. Rank Correlations

Observations Correlated	Sample Composition	No. of Samples	Spearman r_s^a
TBA and peroxide	Cured only	18	0.84
	Uncured only	20	0.91
	Cured and uncured	40	0.60
TBA and rancid odor	Cured only	43	0.82
	Uncured only	25	0.85
	Cured and uncured	32	0.92
Peroxide and rancid odor	Cured only	43	0.80
	Uncured only	24	0.68
	Cured and uncured	32	0.56
TBA and pigment loss	Cured	18	0.87
Peroxide and pigment loss	Cured	18	0.87

^a All values highly significant.

0.5% (Table V) was not protective. Ascorbate alone gave some protection in the one experiment in which it was tried (Table V), but this compound behaves erratically when added to meat, sometimes accelerating rancidity. Hot water extracts of green onion tops and green peppers had no protective effect.

The combination of tripolyphosphate and ascorbate gave excellent protection during the first few months of storage, but the treated samples were highly rancid at 47 weeks (Table I) and 1 year (Table IV). The increase in lipid oxidation is sudden and drastic and may coincide with loss of the ascorbate, although this has not been demonstrated.

BHA was the most effective of the antioxidants tried in preventing lipid oxidation (Table IV). Unfortunately, although no rancid odor was present in the BHA-treated samples even after a year of storage, an unidentified off-odor was evident at least during the last few months of storage. The peculiar "medicinal" odor detracted from the palatability of the cured meat. Further investigation of this problem is needed before BHA can be recommended as an antioxidant under these conditions.

Cured meat protected either with BHA or with tripolyphosphate plus ascorbate (up to the time of the sudden increase in oxidation) showed extremely high peroxide to TBA ratios. These antioxidants appear to prevent peroxide decomposition. Privett (9) has offered evidence that the synergistic activity of ascorbate may be due, at least in part, to its ability to delay peroxide decomposition.

Sensory Scores and Pigment Changes. Statistical evaluations of the correlation between several of the tests used were obtained using the Spearman rank correlation (17). The results are summarized in Table VII.

The correlation of rancid odor with TBA numbers is very high. This is true both for cured and for uncured samples,

and the correlation increases further when the total number of cured and uncured samples are ranked together. When it is considered that the organoleptic ratings were made over a period of many months, with no standards for comparison, a correlation coefficient of 0.92 is surprisingly high.

Peroxide number also correlates well with sensory scores in cured samples. The correlation coefficient is lower when uncured samples are ranked and, as might be expected, drops still more when cured and uncured samples are lumped together. Peroxides appear to be a less useful measure of rancidity than the TBA test, especially when sample treatments affect peroxide stability. The difficulty of designating a "threshold" peroxide number for rancid odor is evident when sensory scores are compared with peroxides in cured and uncured meats.

To correlate pigment losses in cured meats with rancidity tests, pigment ratios in Tables II, IV, and V were calculated for ranking purposes as per cent retention, assuming that a ratio of 1.3 represents complete pigment destruction. These results could then be combined with pigment retentions by the Hornsey method (Table I). Pigment loss showed a high degree of correlation with both the TBA and peroxide numbers. Any antioxidant treatment which retarded lipid oxidation also retarded pigment loss.

All samples in Experiment 1 gave positive sulfhydryl tests throughout a year of storage. Freezing has been shown to protect sulfhydryl groups (4). Free nitrite was present in the cured sample throughout but rapidly disappeared from the cured sample containing ascorbate. This observation is in agreement with earlier work (8).

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